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PRELIMINARY REPORT ON HOG-CHOLERA VIRUS PROPAGATION IN RABBITS, (U)
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DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL INTELLIGENCE AND INFORMATION AGENCY
WASHINGTON, D.C. 20314

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USAMIIA TRANSLATION

Number:

14

USAMIIA-K-7021

Date Completed:

11

28 Feb 1977

12 7p.

Language:

Chinese

Geographic Area:

English Title:

6

PRELIMINARY REPORT ON HOG-CHOLERA VIRUS PROPAGATION
IN RABBITS

Foreign Title:

Author:

10

Li-jang/Ch'in ~~and~~ Ch'ing-tan/Cheng

Source Document:

Journal of the Agricultural Association of China,
No 186

Pages Translated:

pp. 39-45

Publisher:

Date/Place Publication:

March 1948

Distribution Statement:

Document is in the public domain

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409 112

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Accession in

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DDC	Ref Section	<input type="checkbox"/>
UNANNOUNCED		<input type="checkbox"/>
JUSTIFICATION		<input type="checkbox"/>

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PRELIMINARY REPORT ON HOG-CHOLERA VIRUS PROPAGATION IN RABBITS

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Agriculture and Forestry

JOURNAL OF THE AGRICULTURAL ASSOCIATION OF CHINA, NO 186, MARCH 1948, pp 39-45
(Created in the Seventh Year [1918] of the Republic)

1. Introduction

In the past twenty years or so, as a result of exerted efforts of virologists, our knowledge concerning virus diseases has improved considerably and the field of virology has been advancing everyday. Some viruses that had been regarded to be capable of being propagated in specific hosts only, such as rinderpest virus (1,7,0,10,) Colorado tick fever virus (5) and dengue fever virus (4,) etc. have now gradually been cultured and propagated in chick embryos or other non-specific hosts. The significance of these achievements is not limited to the factor of propagation, because a new pathway has thus been opened with respect to techniques of disease prevention and control in veterinarian medicine. For example, rinderpest vaccines, with proven effectiveness, cultivated through chick embryos or domestic rabbits, are very inexpensive to manufacture. The creation of these vaccines should, of course, be credited to the successful efforts in propagating these viruses in non-specific hosts.

Formerly, hog-cholera virus was regarded as not being able to reproduce in the body of non-specific hosts. Samples of failed attempts are too numerous to mention. It was reported, however, that in 1939 Zichis (2) was able to infect sheep with hog-cholera virus, passing through as many as ten generations. Subsequently, in 1941, Tenbroeck (3) succeeded in inoculating the chorion of chick embryo with crushed tissues of testicles of healthy hogs with hog-cholera virus mixed in. The virus infection passed through twelve generations and remained extremely potent. Since then, there had not been reports of successful attempts until 1946, however. In that year, inspired by the successful propagation of rinderpest virus in rabbits, Korprowski (6) and Baker (7,8) tried to cultivate hog-cholera virus in rabbits. After repeated failures and consequent revisions of their experimental techniques they finally succeeded in propagating hog-cholera virus in rabbits. On the basis of this phenomenon of success, the possibility of manufacturing economical and effective hog-cholera vaccine was immediately predicted.

Hog-cholera virus is one of the serious veterinary problems in China. Numerous pigs die of this disease every year. In 1947, for example, more than thirty thousand pigs died during the hog-cholera virus epidemic in Chin-hua, Chekiang Province. It amounted to a great economic disaster for the people of that region. Formerly, only the Kabete strain of rinderpest virus was successfully propagated in chick embryo while attempts of using other strains, including the Chinese and the Phillipine strains had all failed. The question of whether or not the characteristic of strain specificity exists in hog-cholera virus as well therefore arises and awaits immediate attention. Hence, an experiment was designed and conducted to propagate the Chinese strain hog-cholera virus in rabbits to test the possibility of adaptation for the purpose of finding a new and convenient technique of preventing and controlling hog-cholera virus disease. A report of the results of the preliminary attempts of this experiment is presented here in this paper for the reference of the authors' colleagues.

During the experiment, encouragements from the Chief of the Central Animal Husbandry Experiment Center, Mr. CH'ENG Shao-chiung [4453 4801 6608] and valuable assistance from Messrs. FANG Kai [2455 7101], TOU Yin-mei [4535 5593 2734], LI Yen-hua [2621 3508 5478], and CHAO Ting-nan [6392 1353 5478] were received. The authors wish to express their appreciation and thanks to them.

II. Materials and Method

The hog-cholera virus used in the experiment was originally obtained by the Central Animal Husbandry Experiment Center through isolation from diseased pigs of hog-cholera virus in the countryside of Yung-ch'ang of Szechwan Province, and stored by passing it through a number of pigs. For this experiment, the aseptic surgical blood-letting technique was employed to kill normal pigs that had been infected with the hog-cholera virus when the pig's body temperature reaction reached the climax. The spleen was removed and immediately placed in a refrigerator before it was taken from the refrigerator to be ground in a sterilized tissue grinder. The ground tissues were diluted with saline water to a ratio of 1/10. This solution was then placed in a centrifugal machine for 10 minutes at a speed of 10,000 revolutions per minute. The clear fluid on the top was sucked up and the residue at the bottom was thrown away. The clear fluid was filtered through a seitz filter; before it was regarded as hog-cholera virus it was inspected and certified to be bacteria-free.

The pigs used in this experiment were purchased locally in the countryside of Nanking. They weighed about 30 chin each. After the pigs arrived they were placed in two sanitary sties for two weeks of observation. The body temperature was taken twice daily and only when there was no abnormal change were they accepted for use in the experiment. After the pigs were inoculated with 4cc of hog-cholera virus that had been passed through rabbits, they were housed in individual pig sties that had never before been used to raise pigs. Each pig was in strict isolation to remove all possibilities of being infected by hog-cholera virus in the environment. Their body temperature was taken twice daily and the details of the condition of disease development were recorded.

With regard to the healthy rabbits used in the experiment, most of them were taken from the Small Animal Laboratory of the Central Animal Husbandry Experiment Center and a portion of them were purchased from a nearby rabbit farm. The varieties included the native, the Himalayan, and the Angora. Their size varied with a body weight of between 1.5 to 5 lbs. There were more males than females.

For the first passing-through of hog-cholera virus in the rabbit body, 2cc of 1/10 diluted filtered fluid of spleen of pigs of hog-cholera virus disease was injected in the vein of the rabbit's ear. After the inoculation, the body temperature was taken every two hours. When the body temperature rose to above 104°F, an aseptic syringe was used to withdraw blood from the heart and 4cc of it was immediately injected hypodermically into a pig; simultaneously, 2cc of it was injected into the second generation rabbit also through the vein of the ear. When the body temperature of the pigs or the rabbits rose as a reaction to the inoculation, the blood of the heart was immediately withdrawn and used to inoculate the rabbits and the pigs of the next generation to form alternative transmission and mutual infection. The purpose of this procedure was not only to test the virulence of the virus but also to promote adaptation of the virus to propagate in the body of rabbits.

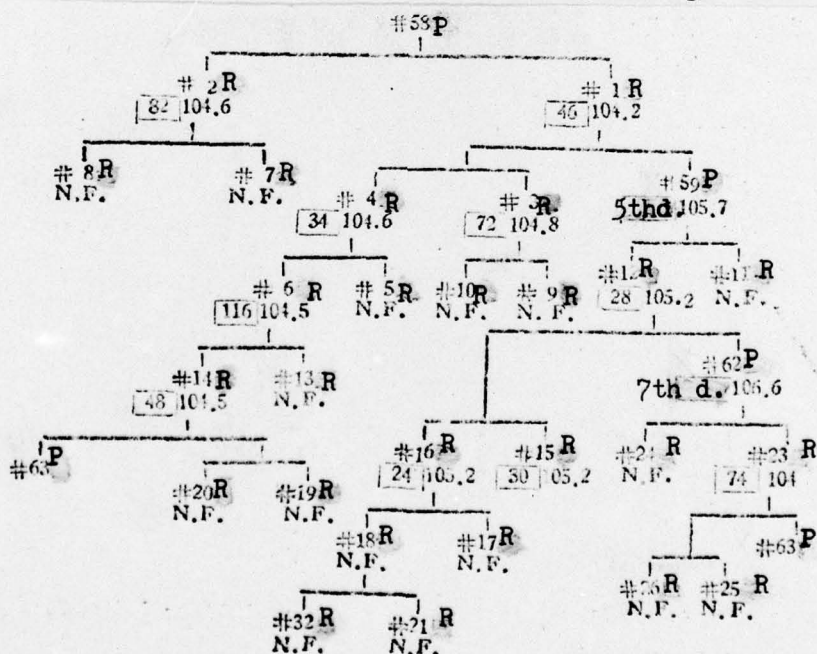
III. Results of Experiment

A total of four pigs and twenty-six rabbits were used as test animals with the aforementioned experimental technique. In order to clarify the condition of the transmission process, a table is included for description (see following page.)

At the beginning of the experiment 2cc of the 1/10 filtered spleen fluid of Pig-No-58 was used to inject into the veins of the ears of Rabbit-No-1 and No-2. Following inoculation, both rabbits appeared to be depressed but they became normal again two hours later. With respect to Rabbit-No-1, its body temperature rose to 104.2°F forty-six hours later; the blood of the heart was withdrawn and used to inoculate Pig-No-59 and Rabbits No-3 and No-4. Three days following the inoculation, Pig-No-59 began to develop a fever, reaching the climax (105.7°F) on the fifth day. After test was made of the blood to prove it to be bacteria-free, 2cc of the blood of the heart was used to inject into the vein of the ears of Rabbits No-11 and No-12. Later, the pig died on the tenth day; pathological changes of hog-cholera virus were found in dissection. The body temperature of Rabbit No-12 rose very fast eighteen hours following inoculation (105.2°F); 2cc of the blood of its heart was withdrawn to inoculate Pigs No-62 and Rabbits No-15 and No-16. Rabbits No-15 and No-16 demonstrated no reaction after the inoculation, but the body temperature of the Pig No-62 began to elevate on the fourth day after the inoculation. On the eighth day, Pig No-62 died and autopsy revealed pathological changes of hog-cholera virus. For the purpose of proving it to be in fact hog-cholera virus, a healthy pig was inoculated with the virus so as to positively determine that Pig No-62 did indeed die of hog-cholera beyond the slightest doubt. The blood of the heart of Pig No-62 was withdrawn on the

seventh day and used to inoculate Rabbits No-23 and No 24. Of the two, the body temperature of Rabbit No-23 rose to 104°F at seventy-four hours and its blood was withdrawn to inoculate Pig No-63 and Rabbits No-25 and No-26, which were observed for five days, but no fever reaction was detected. The Pig No-63 had been inoculated with the blood of Rabbit No-14 before it was inoculated with the blood of the heart of the Rabbit No-23, but there was no reaction in both instances. At first, it was thought to be naturally immune, but later when it was injected with virus of strong potency symptoms of hog-cholera virus developed. The suspicion of natural immunity was thus eliminated. The loss of virulence should have begun with Rabbit No-23. With respect to Rabbits No-3 and No-4, after having been inoculated with the blood of Rabbit No-1, the body temperature of Rabbit No-4 rose to 104.6°F at thirty-four hours following the injection; the blood of its heart was thus used to inoculate Rabbits No-5 and No-6. Rabbit No-6 continued to transmit to Rabbits No-13 and No-14. Of these, the body temperature of Rabbit No-14 rose to 104.5°F forty-eight hours afterwards, but inoculation of its blood in Pig No-63 and Rabbits No-19 and No-20 brought no reaction at all to indicate that virulence had been lost. Test result of Pig No-63 with the blood of this rabbit was described previously. Rabbit No-3 reacted with a slight elevation of body temperature (104.5°F) as late as seventy-two hours after the inoculation, but inoculation of the blood of the heart of Rabbit No-3 in Rabbits No-9 and No-10 brought no reaction in both cases.

Table 1 Condition of Hog-Cholera Virus Passing Through Rabbits



Note: P - pig; R - rabbit; d. - day; - number of hours following inoculation when the body temperature rose; 104... - degree F of body temperature when the blood was withdrawn; N.F. - no fever reaction.

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With respect to Rabbit No-2, its body temperature began to elevate (104.6°F) as late as eighty-two hours after the inoculation. Its blood was injected into Rabbits No-7 and No-8, but no fever reaction was detected. The test was thus abandoned.

Of the twenty-six rabbits inoculated in the experiment, ten had reactions, amounting to about two fifths. Immediate symptom following inoculation /of all the rabbits/ was the appearance of slight depression but all recovered from the depressed state in two to six hours; but with respect to the ten rabbits the body temperature was elevated to demonstrate their reaction to the inoculation.

IV. Discussion and Conclusion

The preliminary experiment of the transmission of hog-cholera virus in rabbits may be briefly summarized as demonstrating the following notable results:

- (1) With alternative transmission technique, hog-cholera virus was successfully passed through the bodies of rabbits to prove that that virus is positively capable of infecting rabbits, through as many as four generations without reduction of its virulence.
- (2) By using hog-cholera virus passing through rabbits alone, virulence was transmitted for four generations, but when the blood of the heart of the fourth generation rabbit was used to infect pigs, the attempt was not successful. Virulence of the virus appeared to have been lost.
- (3) After the rabbits were infected by inoculation, the shortest incubation period was twenty-four hours while the longest was one hundred sixteen hours, but if the reaction time was more than seventy hours following inoculation, virulence appeared to be difficult to pass through.
- (4) The rate of hog-cholera virus infection in rabbits appeared to be two fifths. With regard to varieties, this experiment used fourteen Himalayan, four Angora, and three native varieties. Of these three Himalayans, four Angorans, and three natives reacted to the injections. It appeared that the native strain was the easiest to infect, followed by the Angora, with the Himalayan relatively more difficult. The number of rabbits used in the experiment remains to be very few; further experiment is needed to determine this factor.
- (5) With regard to inoculation transmission of hog-cholera virus in non-specific hosts, there appeared to be no strain specificity, unlike the case of rinderpest virus, only the Kabete strain of which had been found to be capable of growing in chick embryos.

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